GENOMIC TEST REPORT

PATIENT INFORMATION & SAMPLE DETAILS

Patient Name	Mrs. BHAKTI KULKARNI	**Age/Gender**	39Y/F
Sample Type	WB-EDTA	**Ref. Doctor**	Dr. ANURADHA SOWANI
Date of Sample Receipt	28/02/2025	**Date of Report**	25/03/2025
Clinical Condition for Testing	**BRCA1 & BRCA2 testing**		
Genomic Sample ID			

CLINICAL SUMMARY

Mrs. Bhakti Kulkarni, a 39-year-old female has been referred for genetic screening of ***BRCA1*** and ***BRCA2*** genes, in view of a strong family history of cancers.

TEST RESULT

NEGATIVE for Pathogenic/Likely pathogenic variants associated with indicated phenotype.

VARIANT DETAILS RELATED TO INDICATION FOR TESTING

BRCA1 & ***BRCA2*** genes have been primarily screened from the Whole exome panel, which consists of ~22,000 genes.

SINGLE NUCLEOTIDE VARIANT (SNV):

- No pathogenic/ likely pathogenic/ high impact SNVs identified.

COPY NUMBER VARIANTS (CNVs):

- No pathogenic/ likely pathogenic/ high impact CNVs identified.

SECONDARY FINDINGS

- No pathogenic variants in genes on the ACMG recommended secondary list were detected.

Note: We adhere to ACMG guidelines for disclosing incidental findings in whole exome sequencing. We communicate notable pathogenic or likely pathogenic variants in specified genes related to recommended phenotypes, contingent upon the informed consent.

Mrs. Bhakti Kulkarni
Sowmya

SEQUENCING DETAILS

Sequencing Type	Germline
Encoding	Illumina 1.9
Sequence Length	151bp
Mean Sequencing Depth	120x
Overall Alignment rate	99.98%
Q30 score	95.14%

BIOMARKERS EVALUATED

BRCA1* and *BRCA2

SUGGESTIONS

- **After the age of 50 years, PAP smear, Mammogram and a gynecologist follow up every year is recommended.**
- **Follow a healthy diet (Anti Inflammatory diet principles).**
- **Regular physical activity and healthy lifestyle.**
- Interpretation of this result should be done in context of a patient's medical record, family history and biochemical profile.
- Please note that interpretation and classification of the variants reported here may change overtime.
- Genetic counselling is advised for services regarding the implications of these results.

TEST DESCRIPTION & METHODOLOGY

Variants associated with the reported clinical condition are analyzed by Whole Exome Sequencing (WES) using the Next Generation Sequence (NGS) analyzer – Illumina's NovaSeq 6000. The DNA libraries were prepared using Roche KAPA hyper Exome panel and sequenced to a mean depth of >80-100x coverage. The panel works on a principle of probe hybridization using DNA probes of around 120mer length providing high and specific interactions with target DNA molecules along with - dual molecular barcodes with dual sample indexing for library preparation. The usage of these dual molecular barcodes reduces or eliminates the index hopping and false positives thus making it more reliable. The NGS test is performed with >95% coverage at a higher depth. Recommended read length of 2 x 150 bps is considered for WES panel sequencing and paired end reads are considered for analysis. Huge quantities of NGS based omics data is concised for better clinical guidance. The raw data with Q30≥80% is considered for analysis, checked for quality by fastQC and aligned to the human reference genome GRCh38.

Variant identification and interpretation are critical steps in making genetic diagnosis and personalized medicine a reality. GATK guidelines are followed for variant identification. The variants were annotated and filtered using the in-house and commercial analysis workflows. Interpretation of sequence variants strictly adheres to the latest ACMG guidelines. In the assessment of the variant classification, GenepoweRx considers information and evidence that includes, but is not limited to, the following 5 significant parameters. The functional impact of the gene in causing the disease phenotype, functional impact of variation in the gene product based on in silico, in vitro, and in vivo studies, variant-disease association, prevalence and significance. This includes comparison against the gnomAD population catalog of variants in 123,136 exomes, the 1000 Genomes Project Consortium's publication of 2,500 genomes, the NCBI ClinVar database of clinical assertions on variant's pathogenicity and multiple lines of computational evidence on conservation and functional impact. The test results are then carefully reviewed and manually curated by our team of highly trained and experienced genome analysts.

Variant Assessment Process

The following databases and algorithms are used to annotate and evaluate the impact of the variant in the context of human disease: 1000 genomes, gnomAD, ClinVar, OMIM, dbSNP, NCBI RefSeq Genes, ExAC Gene Constraints, VS-SIFT, VS-PolyPhen2, PhyloP, GERP++, GeneSplicer, MaxEntScan, NNSplice, PWM Splice Predictor. Analysis was reported using HGVS nomenclature (www.hgvs.org/mutnomen) as implemented by the VarSeq transcript annotation algorithm. The reported transcript matches that used most frequently by the clinical labs submitting to ClinVar.

Variant Impact (from ensembl) indicates the strength of the variant based on a variant's probability to alter protein structure or function and its effect on the phenotype. The variant Impact is obtained from ensembl, which is calculated by a rule based approach to predict the effects that each allele of the variant may have on each transcript. The set of consequence terms, defined by the Sequence Ontology (SO), that can be currently assigned to each combination of an allele and a transcript is shown in the table below. Note that each allele of each variant may have a different effect in different transcripts. Variant Impact is classified into 4 groups

High: This variant is assumed to have (disruptive) impact in the protein, probably causing protein truncation, loss of function or triggering nonsense mediated decay.

Variant types included under this category: Transcript ablation- complete deletion of functional region of the gene; Splice acceptor variant; Splice donor variant; Stop gained; Frameshift variant; Stop lost; Start lost; Transcript amplification.

Moderate: A non-disruptive variant which can change protein effectiveness.

Variant types included under this category: Inframe insertion; Inframe deletion; Missense variant; Protein altering variant.

Low: Assumed to be mostly harmless and unlikely to change protein behavior.

Variant types included under this category: Splice region variant; Incomplete terminal codon variant; Start/Stop retained variant; Synonymous variant.

Modifier: Usually non-coding variants where predictions are difficult or there is no sufficient evidence of impact.

Variant types included under this category: Mature miRNA variant; UTR region Variants, TF binding site variant; Regulatory region variant, Intergenic variant, and variants of many other location where's there's no sufficient evidence.

Ref: https://asia.ensembl.org/info/genome/variation/prediction/predicted_data.html

ANNOTATION SOURCES

This interpretation was preformed using the following annotation sources:

Name	Version	Type
gnomAD Exomes Variant Frequencies 2.0.1, BROAD	2017-05-09	Frequency
1kG Phase3 - Variant Frequencies 5a with Genotype Counts, GHI	2015-05-26	Frequency
Multiple Sequence Alignments of 100 Vertebrates, UCSC	2015-05-06	Annotation
CADD Scores 1.6	1.6	Annotation
Reference Sequence GRCH37g1k V2, 1000Genomes	2022-10-17	Annotation
ClinVar CNVs and Large Variants 2024-01-04, NCBI	2024-01-04	Annotation
dbSNP 155, NCBI	2021-05-25	Annotation
Haploinsufficiency Predictions Version 3, DECIPHER	v3	Annotation
DECIPHER Population CNV v9.2	2015-09-15	Annotation
Genomic Super Dups 2014-10-19, UCSC	2014-10-19	Annotation
GnomAD High Frequency CNV Regions 2019-11-25, GHI	2019-11-25	Annotation
gnomAD Structural Variants 2.1, BROAD	2019-03-06	Annotation
DGV CNVs - Gold Standard Variants 2016-05-15 v3, DGV	2016-05-15 v3	Annotation
1kG Phase3 CNVs and Large Variants 5b V2, GHI	2015-08-18v2	Annotation
Missense Badness and MPC, BROAD	2017-07-14	Annotation
Low Complexity Regions and Universal Mask-GHI	2015-03-29	Annotation
Repeating Elements by RepeatMasker, UCSC	2014-04-06	Annotation
Genetics Home Reference 2024-02-06, GHI	2024-02-06	Annotation
Clinical Genomic Database 2023-12-01, GHI	2024-01-02	Annotation
Reference Sequence GRCh38 V2, NCBI	2022-10-17	Annotation
ClinGen Gene Dosage Sensitivity 2024-03-01, NCBI	2024-03-01	Annotation
ClinGen Region Dosage Sensitivity 2024-03-01, NCBI	2024-03-01	Annotation
ClinVar Assessments 2024-01-04, NCBI	2024-01-04	Annotation
ClinVarCNVsandLargeVariant Assessments 2024-01-04, NCBI	2024-01-04	Annotation
ClinVar 2024-01-04, NCBI	2024-01-04	Annotation
ClinVar Transcript Counts 2024-01-04, NCBI	2024-01-04	Annotation
Conservation Scores Exonic, GHI	2020-10-02	Annotation
DECIPHER Developmental Disorders 2024-03-01, GHI	2024-03-01	Annotation
gnomAD - Gene Constraint 2.1.1 v2, BROAD	2019-03-06 v2	Annotation
Gene Identifiers and Descriptions 2022-12-19, GHI	2022-12-19	Annotation
Human Phenotype Ontology 2023-11-20	2023-11-20	Annotation
SIFT and PolyPHen2 Missense Predictions 2021-04-21, GHI	2021-02-11	Annotation
MONDO 2023-11-17, GHI	2023-11-17	Annotation
Mondo Gene Disease Association 2023-11-21, MI	2023-11-21	Annotation
OMIM Genes 2024-03-08, GHI	2024-03-08	Annotation
Orphanet Gene Associations 2024-02-01, GHI	2024-02-01	Annotation
Cytobands 2014-06-11, UCSC		Annotation
RefSeq Genes 110, NCBI	2022-04-12	Annotation
InterPro Regions 2024-02-08	2024-02-08	Annotation
ClinGen Gene Disease Validity 2024-02-06, NCBI	2024-02-06	Annotation

Glossary of Genetic Terms Used in the Report

Variant	A variant is refers to genetic alteration or differences in the DNA sequence at a specific position in an individual's genome.
Pathogenic	A variant which is more likely to cause a disease. All the variants which can cause loss of Protein function or predictable significant damage to the gene product or can alter protein/protein interactions fall under this category.
Likely Pathogenic	A variant which is strongly suspected to contribute to disease development, but conclusive scientific evidence is currently lacking. Further research is anticipated to substantiate its potential pathogenicity.
Variant of Uncertain significance (VUS)	The variant presents challenges in definitive classification as either pathogenic or benign due to limited current scientific evidence. Additional testing of the patient or family, as advised by the clinician, may be necessary. Significance assessment is likely contingent on evolving scientific evidence over time.
Risk variant	A variant which can slightly increase the risk of the disease predisposition.
Benign	A genetic change that could be common in the general population. These are not known to cause the disease directly but can have a cumulative effect when multiple benign variants are present in important regions of the genome.
Homozygous	Every person has two copies of same chromosome or DNA. Homozygous mutant refers to having mutated or altered DNA in both the copies of the gene.
Heterozygous	Indicates 2 non identical or mismatched copies of a gene. i.e One normal copy and one mutated copy. The mutations can be Inherited from the parents or acquired (due to various exposures) during one's lifetime.

CLASSIFICATION SYSTEM AND FREQUENCY THRESHOLDS

This interpretation was preformed using the ACMG variant classification guidelines. The following recessive gene thresholds were used:

- Common Allele Frequency: 0.01
- High for Disorder Allele Frequency: 0.0015
- Extremely Rare Allele Frequency: 0.0002

The following dominant gene thresholds were used:

- Common Allele Frequency: 0.005
- High for Disorder Allele Frequency: 0.0005
- Extremely Rare Allele Frequency: 0.0001

Sub-populations were excluded from consideration if the total allele number failed to exceed 2000.

ACMG variant classification guidelines (PMID: 25741868)

Benign ← → Pathogenic

	Strong	Supporting	Supporting	Moderate	Strong	Very Strong
Population Data	MAF is too high for disorder *BA1/BS1* **OR** observation in controls inconsistent with disease penetrance *BS2*			Absent in population databases *PM2*	Prevalence in affecteds statistically increased over controls *PS4*	
Computational And Predictive Data		Multiple lines of computational evidence suggest no impact on gene /gene product *BP4* Missense in gene where only truncating cause disease *BP1* Silent variant with non predicted splice impact *BP7*	Multiple lines of computational evidence support a deleterious effect on the gene /gene product *PP3*	Novel missense change at an amino acid residue where a different pathogenic missense change has been seen before *PM5* Protein length changing variant *PM4*	Same amino acid change as an established pathogenic variant *PS1*	Predicted null variant in a gene where LOF is a known mechanism of disease *PVS1*
Functional Data	Well-established functional studies show no deleterious effect *BS3*		Missense in gene with low rate of benign missense variants and path. missenses common *PP2*	Mutational hot spot or well-studied functional domain without benign variation *PM1*	Well-established functional studies show a deleterious effect *PS3*	
Segregation Data	Non-segregation with disease *BS4*		Co-segregation with disease in multiple affected family members *PP1*	Increased segregation data →		
De novo Data				*De novo* (without paternity & maternity confirmed) *PM6*	*De novo* (paternity & maternity confirmed) *PS2*	
Allelic Data		Observed in *trans* with a dominant variant *BP2* Observed in *cis* with a pathogenic variant *BP2*		For recessive disorders, detected in *trans* with a pathogenic variant *PM3*		
Other Database		Reputable source w/out shared data = benign *BP6*	Reputable source = pathogenic *PP5*			
Other Data		Found in case with an alternate cause *BP5*	Patient's phenotype or FH highly specific for gene *PP4*			

Criteria to Classify Sequence Variants

Pathogenic	**Likely Pathogenic**
1 Very Strong (PVS1) AND ≥1 Strong (PS1–PS4) OR ≥2 Moderate (PM1–PM6) OR 1 Moderate (PM1–PM6) and 1 Supporting (PP1–PP5) OR ≥2 Supporting (PP1–PP5) ≥2 Strong (PS1–PS4) OR 1 Strong (PS1–PS4) AND ≥3 Moderate (PM1–PM6) OR 2 Moderate (PM1–PM6) AND ≥2 Supporting (PP1–PP5) OR 1 Moderate (PM1–PM6) AND ≥4 Supporting (PP1–PP5)	1 Very Strong (PVS1) AND 1 Moderate (PM1–PM6) OR 1 Strong (PS1–PS4) AND 1–2 Moderate (PM1–PM6) OR 1 Strong (PS1–PS4) AND ≥2 Supporting (PP1–PP5) OR ≥3 Moderate (PM1–PM6) OR 2 Moderate (PM1–PM6) AND ≥2 Supporting (PP1–PP5) OR 1 Moderate (PM1–PM6) AND ≥4 Supporting (PP1–PP5)
Benign	**Likely Benign**
1 Stand-Alone (BA1) OR ≥2 Strong (BS1–BS4)	1 Strong (BS1–BS4) and 1 Supporting (BP1–BP7) OR ≥2 Supporting (BP1–BP7)

*Variants are classified as Uncertain Significance if other criteria are unmet or the criteria for benign and pathogenic are contradictory.

REFERENCES

PMID	CITATION
25741868	Richards S, Aziz N, Bale S, et.al.; ACMG Laboratory Quality Assurance Committee. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015 May;17(5):405-24. doi: 10.1038/gim.2015.30. Epub 2015 Mar 5.
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10447503	Sherry ST, Ward M, Sirotkin K. dbSNP-database for single nucleotide polymorphisms and other classes of minor genetic variation. Genome Res. 1999 Aug;9(8):677-9.
19344873	Firth HV, Richards SM, Bevan AP, Clayton S, Corpas M, Rajan D, Van Vooren S, Moreau Y, Pettett RM, Carter NP. DECIPHER: Database of Chromosomal Imbalance and Phenotype in Humans Using Ensembl Resources. Am J Hum Genet. 2009 Apr;84(4):524-33. doi: 10.1016/j.ajhg.2009.03.010. Epub 2009 Apr 2.
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LIMITATIONS and DISCLAIMER

- This report is provided as an information source for clinicians and is not intended to be considered a substitute for professional medical advice. If you have or suspect you have a medical problem, contact your clinician for personalized treatment or therapy.
- Partial reproduction of this report is not advisable and should be avoided
- The genomic information needs to be correlated clinically. Only pathogenic and likely pathogenic variant information is provided in the report. VUS if found, are reported only when relevant pathogenic and likely pathogenic variants are not seen. However, these details of the relevant VUS and benign variants will be provided upon request if required.
- The translation of genomics knowledge and data into the current report requires generalization and continuous expansion of genomic insights from the literature. Hence minor errors are anticipated.
- This report and usefulness of the information provided in the report shall not warranty or hold any liability or responsibility for any direct, indirect, incidental, consequential indemnities arising out of the use of, or inability to use the information. This doesn't hold any claims for medico-legal reasons also.
- This report makes no promises or guarantees that the reported condition/s would develop anytime. Other genetic, environmental, and clinical factors might influence the patients' phenotypic response to the condition.
- Proper understanding of the risks aids in better management of the metabolic conditions with traditional therapies or other treatment options and helps in prevention/delay of the disease or making it less harmful.
- Normal findings do not rule out the diagnosis of a genetic disorder since some genetic abnormalities may be undetectable with this test.
- The transcript used for clinical reporting generally represents the canonical transcript (MANE Select), which is usually the
- longest coding transcript with strong/multiple supporting evidence. Variants annotated on incomplete and nonsense mediated decay transcripts will not be reported.
- This test cannot reliably detect mosaicism. Some genes have inherent sequence properties (for example: repeat, homology, or pseudogene regions, high GC content, rare polymorphisms) that may result in suboptimal data, and variants in those regions may not be reliably identified.
- The genomic variations reported may include somatic and germline variations; but does not distinguish between the two alterations.
- The chance of a false positive or false negative result due to laboratory errors incurred during any phase of testing cannot be completely excluded, though very rare. The mutations are not validated/reflex tested.
- Variants reviewed by expert panel and submitted by multiple research groups are only considered for reporting. Variants with insufficient evidence are excluded from the report.
- Reflex testing is mandatory when CNVs are reported. MLPA and/or array CGH, or other targeted approaches, are generally recommended as follow-up tests to ensure accurate CNV characterization, given the inherent differences in technologies such as resolution, sensitivity, and target specificity. Further clinical correlation with the patient's phenotype and additional testing may be required for a complete interpretation.

- Genetic testing is generally highly accurate, but occasional inaccuracies may arise due to errors in reporting clinical/medical information. Interpretations are made with the assumption that any information provided on medical and family history is accurate. Consultation with a genetics professional is recommended for better interpretation of results.
- VUS mentioned in the report require additional correlation with clinical phenotype, other investigation reports, and segregation analysis in family members. We are not liable for any inappropriate interpretation, communication, clinical actions, or reproductive decisions based on reported VUS. The classification of VUS may change as the clinical phenotype evolves or with the availability of more information in scientific literature/databases.
- Collection, processing, use, storage and retention of the anonymized data from the tests conducted are used for ongoing test developments, educational, scientific research and/or other related activities.

This test was developed, and its performance characteristics were determined by GenepoweRx. This test has not been cleared or approved by the U.S. Food and Drug Administration (FDA). The FDA has determined that such clearance or approval is not necessary. These interpretations are based on ACMG/AMP GATK, CPIC Guidelines. This test can be used for clinical screening and research purposes only. The test is registered in CDSCO, India, as Uppalu-Hyder-TE/M/IVD/008407, Class C of Medical Devices, 2022.

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This report is provided as an information source for clinicians and is not intended it to be considered a substitute for professional medical advice. If you have or suspect you have a medical problem, contact your physician for personalized treatment or therapy. The translation of genomics knowledge and data into the current report requires generalization and continuous expansion of genomic insights from literature. Hence, minor errors are anticipated. This genomic report and the usefulness of the information provided in the report shall not warranty or hold any liability or responsibility for any direct, indirect, incidental, or consequential indemnities arising out of the use of or inability to use the information. This report makes no promises or guarantees that the reported condition/s will develop at any time. Other genetic, environmental, and clinical factors might influence the patients' phenotypic response to the condition. Proper understanding of the risks aids in better management of metabolic conditions with traditional therapies or other treatment options and helps in the prevention/delay of the disease or makes it less harmful.

Genetic testing plays a key role in the diagnosis of the root cause of a disease or condition. It provides excellent guidance in deciding the right medical regimen. But sometimes, a few non-treatable variants are also identified. Not all genetic changes affect health. It is difficult to know whether identified variants are involved in the condition of interest. Sometimes, an identified variant is associated with a different genetic disorder that has not yet been diagnosed (these are called incidental or secondary findings). A finding of biomarker alteration does not necessarily indicate the pharmacologic effectiveness (or lack thereof) of any drug or treatment regimen; a finding of no biomarker alteration does not necessarily indicate the lack of pharmacologic effectiveness (or effectiveness) of any drug or treatment. No Guarantee of Clinical Benefit: This Report makes no promises or guarantees that a drug will be effective in the treatment of disease in any patient. This Report also makes no promises or guarantees that a drug with a potential lack of clinical benefit will, in fact, provide no clinical benefit. It is possible that a pathogenic variant is present in a gene that was not selected for analysis and /or interpretation in cases where insufficient phenotypic information is available. Due to inherent technology limitations and constant upgradation of research and literature, not all bases of the exome can be covered. Accordingly, variants in regions of insufficient coverage may not be identified and/or interpreted. Therefore, it is possible that pathogenic variants are present in one or more of the genes analyzed but have not been detected. The variants not detected by the assay that was performed may impact the phenotype. For in vitro research use only. This test must be ordered by a qualified medical professional in accordance with required medical regulations.

Patient care treatment decisions must be based on the self-determining medical judgment of the respective physician. Do consider complete patient information such as patient preferences, medical history and family history, physical examination profiles, and other lab results per the standard of care medical practice. The reported results are for the information of the referring doctor only. It should be noted that this test is restricted to a limited number of genes and does not include all intronic and non-coding regions. This report only includes variants that meet a level of evidence threshold for cause or contribute to disease. More evidence for disease association of genes and causal pathogenic variants is discovered every year, and it is recommended that genetic variants are re-interpreted with updated software and annotations periodically.